APPENDIX C

The Structure of Calcium-Free Human m-Calpain

Implications for Calcium Activation and Function

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The calpains form a growing family of structurally related intracellular multidomain cysteine proteinases containing a papain-related catalytic domain, whose activity depends on calcium. The calpains are believed to play important roles in cytoskeletal remodeling processes, cell differentiation, apoptosis and signal transduction, but are also implicated in muscular dystrophy, cardiac and cerebral ischemia, platelet aggregation, restenosis, neurodegenerative diseases, rheumatoid arthritis and cataract formation. The best characterized calpains, the ubiquitously expressed μ- and m-calpains, are heterodimers consisting of a common 30-kDa small and a variable 80-kDa subunit. The recently determined crystal structures of human and rat m-calpain crystallized in the absence of calcium essentially explain the inactivity of the apoform by catalytic domain disruption, indicate several sites where calcium could bind causing reformation of a papain-like catalytic domain, and additionally reveal modes by which phospholipid membranes could reduce the calcium requirement. Current evidence points to a cooperative interaction of several sites, which, upon calcium binding, trigger the reformation of a papain-similar catalytic domain. (Trends Cardiovasc Med 2001;11:222–229). © 2001, Elsevier Science Inc.

The calpains (E.C 3.4.22.17: Clan CA, family C02) are generally characterized as a family of calcium-dependent cytosolic cysteine proteinases with a papain-resembling catalytic domain. The calpains seem to catalyse the limited proteolysis of proteins involved in cytoskeletal remodeling, signal transduction, cell cycle regulation, cell differentiation, apoptosis and necrosis, embryonic development and long-term potentiation in the central nervous system (see, e.g., Carafoli and Molinari 1998, Ono et al. 1998, Wang 2000). Potential protein substrates are cytoskeletal proteins and membrane proteins known to control various functional processes in response to extracellular stimuli. The calpains are also implicated, however, in various pathophysiological processes, including type-2 diabetes mellitus (Horikawa et al. 2000), muscular dystrophy (Richard et al. 1999), cataractogenesis, inflammation, arthritis, and Alzheimer's and Parkinson's diseases (Vanderklish and Bahr 2000). Furthermore, it is now clear that organ ischemia, trauma and hemor-

rhage can, by increasing the calcium level, lead to an activation of calpain, which in turn may trigger the proteolysis of cytoskeletal proteins, cell membrane proteins and regulatory kinases. Indeed, it has been shown experimentally that blockage of calpain-like proteolytic activities with inhibitors can reduce injuries of the brain (Lee et al 1991, Rami and Krieglstein 1993, Wang et al. 1996, Yokota et al. 1999), the liver (Kohli et al 1997) and the heart (Iizuka et al. 1992, Matsumura et al 1993) caused by ischemia/reperfusion. In recent work, McDonald et al. (2001) show that inhibition of calpain reduces ischemia/reperfusion injury by preventing the expression of transcription factor NF-κB-dependent genes. Some older reports should be considered with some care, however, as most of the inhibitors (such as the calpain inhibitors I and II) are not really calpain-specific.

The "classic" μ- and m-calpains are the best-characterized calpains. They are heterodimers consisting of homologous but distinct (large) L-chains and a common (small) S-chain. On exposure to calcium at concentrations of 5–50 μM (μ-calpain) and 200–1000 μM (m-calpain), both calpains become active in vitro. In vivo, however, where the calcium concentrations are in general far below 1 μM (Goll et al. 1992), the calpain activity might be additionally regulated by other mechanisms such as binding of activators or interaction with phospholipids (Pontremoli et al. 1985, Saido et al. 1992). Currently, at least 12 different calpains have been characterized in mammals, which are "ubiquitously" (such as μ- and m-calpain) or "tissue-specifically" expressed (see Table 1). Only a few (such as the μ- and m-calpains) are heterodimers, while the majority of calpains likely consist of an isolated L-subunit alone. Besides these "typical" calpains, which possess a μ-/m-like L-chain, "atypical" calpains, which, besides a papain-related catalytic domain, contain other amino- and carboxy-terminal domains, have been found in lower organisms such as nematodes, fungi and yeast, but very recently also in the human/mammalian genome (see Sorimachi et al. 1997). The proteolytic activity of most of these calpains depends on the presence of free calcium, while a few other calpains (such as p94: Sorimachi et al. 1989) do not seem to require cal-

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Table 1. Family of currently known human calpain genes, indicating the different names used for their products, their homologues in other organisms, the tissues where they are expressed, and other important characteristics

Gene	Gene product	Homologues	Expression	Note
CAPN1	μ-calpain large subunit (calpain 1)	(Typical calpain)	Ubiquitous	
CAPN2	m-calpain large subunit (calpain 2)	(Typical calpain)		
CAPN3	p94 (nCL-1, calpain 3)	(Typical calpain)	Skeletal muscle	LGMD2A[b]
	Lp82 (a.s.p.)[a]	(Typical calpain)	Lens	
CAPN5	hTRA-3 (nCL-3, calpain 5)	TRA-3 (nematode)	Testis, brain	
CAPN6	Calpain 6 (calpamodulin, CAPNX)	TRA-3 (nematode)	Placenta, embryonic muscle	No Cys at active site
CAPN7	PalBH (calpain 7)	PalB (fungi), Cpl1 (yeast)	Ubiquitous	
CAPN8	nCL-2 (calpain 8)	(Typical calpain)	Stomach	
	nCL-2' (a.s.p.)[a]		Stomach	Ca²⁺-dependent
CAPN9	nCL-4 (calpain 9)	(Typical calpain)	Digestive tracts	
CAPN10	Calpain 10A-H		Ubiquitous	NIDDM[c]
CAPN11	Calpain 11	(Typical calpain)	Testis	67% identical to chicken μ/m-calpain
CAPN12	Calpain 12	(Typical calpain)	Follicle	
(CAPN13)	SOLH	SOL (Drosophila)	Ubiquitous	
CAPN4	μ-, m-calpain small subunit	(SEF hand protein)	Ubiquitous	

[a] Alternative splicing product.
[b] Limb girdle muscle dystrophy 2A.
[c] Non-insulin-dependent diabetes mellitus.

cium for their activity. The only known natural/endogenous inhibitors of activated calpains are calpastatin (Takano et al. 1995) and the second cystatin domain of kininogen (Salvesen et al. 1986).

On the basis of amino acid homologies, the L- and the S-subunit had been originally described as consisting of four domains, I to IV, including a papain-like domain II, and two domains, V and VI, respectively (Imajoh et al 1988, Ohno et al 1986). The recent crystal structures of an S-chain truncated rat m-calpain (Hosfield et al. 1999) and of the full-length human m-calpain (Strobl et al. 2000), determined at 2.7 and 2.3 Å resolution, respectively, in the absence of calcium, have in principle confirmed the proposed multidomain structure (Reverter et al. 2001). These structures have allowed us to explain the inactivity of apo-calpain via a disrupted catalytic domain, have helped to identify several sites where calcium could bind, causing reformation to a functional enzyme, and have given invaluable hints for site-directed mutagenesis experiments to test these hypotheses. Because of the lack of calpain structures determined in the presence of calcium, we currently are forced to speculate about the mechanism of activation and action of calpains.

- Overall Structure of m-Calpain

The m-calpain molecule forms a flat oval disc. Surprisingly, the catalytic domain II, consisting of two subdomains, IIa (gold) and IIb (red), and the calmodulin-like domain pair dIV-dVI (yellow-orange) are not in direct contact with each other but are placed at the upper and the lower poles (in the reference orientation used in Figure 1). Instead, domain III (blue) and the amino-terminal domains I (green) and V (magenta) connect the two calmodulin-like domains with both catalytic subdomains. μ-calpain, which has an identical S-chain and shares with m-calpain a 61% sequence similarity in the L-chain, should have a similar appearance.

The L-chain (see Figure 1) starts with an "anchoring helix" (dI) in a surface cavity of domain VI and continues straight to the "helical subdomain" IIa, clamping both domains together. At Gly19L (mature human m-calpain numbering, with suffixes L and S indicating residues of the large and small subunit, Figure 2), the L-chain joins the catalytic domain, where it forms an outer polar surface shell, before (from Thr93L onward) it folds into the papain-like core. This catalytic domain is broken into two

separate subdomains, with each significantly differing in length and conformation from the equivalent halves in papain (Kamphuis et al. 1984). At the Gly209L-Gly210L "hinge," the L-chain passes over to the "barrel-like subdomain" IIb, forming the typical six-stranded β-pleated sheet rolled into an open barrel and flanked by three additional helices. Despite this barrel having a similar shape as in papain, two of the loops are quite extended. Importantly, the substrate-binding cleft of apo-calpain is parted to form a wide gap and its Cys105L-His262L-Asn286L active-site triad is disrupted, which is incompatible with productive binding and cleavage of peptide substrates (Figure 3).

The L-chain then turns through an open loop before forming the central domain III (Figure 1), with this domain consisting essentially of two opposing four-stranded antiparallel β-sheets and exhibiting the tertiary fold of a compact β-sandwich. Its topology faintly resembles that of so-called C2 domains that are known to occur in a large variety of proteins involved in intracellular signaling (like phospholipase A2, phospholipase C, and protein kinase C) and membrane trafficking (like synaptotagmin: for reviews see, e.g., Rizo and Sudhoff
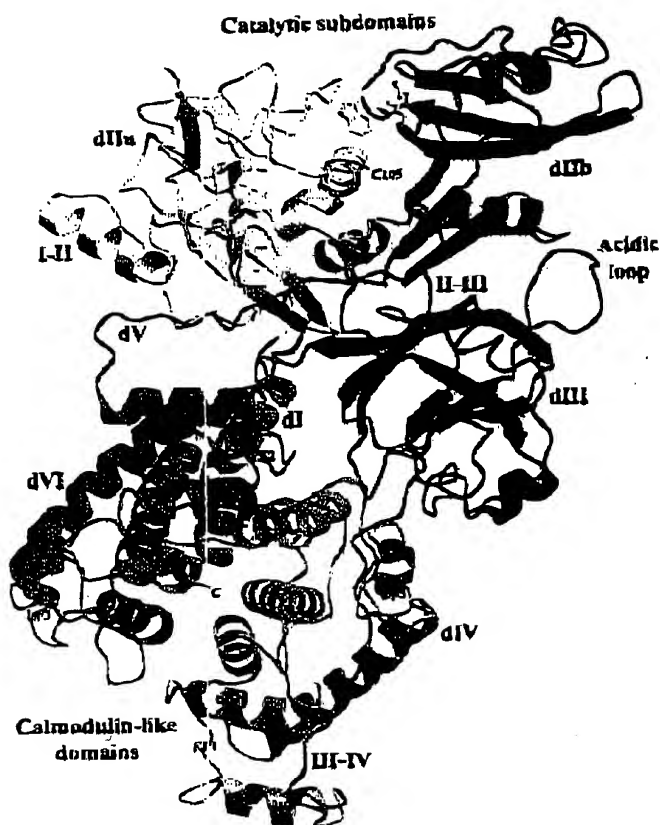
Figure 1. Ribbon structure of the apoform of human m-calpain, shown in reference orientation (Strobl et al. 2000). The 80-kDa L-chain starts in the molecular center (green, dI), folds into the surface of subdomain IIa (gold, I-II linker), forms the helical (gold, dIIa) and the barrel-like (red) subdomains IIa and IIb, descends through the open II-III loop (red), builds domain III (blue), runs down (magenta, III-IV) and forms the right-side calmodulin-like domain IV (yellow). The 30-kDa S-chain becomes visible from Thr95S onward (magenta, dV) before forming the calmodulin domain VI (orange). The catalytic residues Cys105L, His262L, and Asn286L together with Trp288L (top) are shown with all non-hydrogen atoms. The figure was made with MOLSCRIPT (Kraulis 1991) and Raster3D (Merrit and Bacon 1997).

glycine residues, is in human full-length m-calpain represented by clear electron density from Thr85S onward, but may be arranged on the front side of m-calpain. The visible part of domain V runs to the molecular periphery, where it enters the second calmodulin-like domain VI This domain, similarly equipped with five potential EF-hand motifs, together with domain IV forms a quasi-symmetrical heterodimer. A topological comparison shows that the human m-calpain dIV-dVI heterodimer determined in the absence of calcium exhibits an architecture similar to that of the dVI-dVI homodimer from rat m-calpain (Blanchard et al. 1997), in its calcium-free as well as in its calcium-laden form.

- **Probable Structure of the Intact Catalytic Domain**

As mentioned above, the catalytic domain of calcium-free calpain is disrupted. The overall homology with papain strongly suggests that both calpain subdomains IIa and IIb possess the inherent tendency to fuse into a papain-like catalytic domain by optimizing the inter-domain contacts, but are hindered in doing so in the absence of calcium. A conformation similar to the catalytic domain of papain can be achieved by rotating and shifting the barrel-like subdomain IIb against the helical subdomain IIa by more than 50° and 10Å, respectively (Figure 3). After a few rearrangements in the interface, the His262L imidazole side chain would be located beside Cys105L and its hydrogen bond to Asn286L would become shielded from bulk water by the Trp288L indole moiety, as is known for all active papain-like proteinases. As in papain, the dipole moment of the anchoring helix may stabilize the Cys105L thiolate-His262L imidazolium cation and the Gln99 carboxamide nitrogen together with the Cys105L amide nitrogen could form the oxyanion hole.

In this fused catalytic domain, the functional active-site cleft could accommodate a susceptible peptide substrate, and as in papain complexes, the P2 main chain [according to the Schechter and Berger (1967) nomenclature, with P1, P2, etc. and P1', P2', etc. defining substrate residues amino- and carboxy-terminally from the scissile peptide

1998). With respect to the detailed order and connectivity of the eight strands, the calpain domain III differs from these C2 domains, but is similar to each of the three subunits of tumor necrosis factor-α (Strobl et al. 2000). Particularly remarkable in the m-calpain domain III is the solvent-exposed "acidic loop," which carries 10 acidic residues within its 11-residue Glu392L–Glu402L segment (Figures 1 and 2). This loop is spatially adjacent to subdomain IIb and certainly interacts electrostatically with the many positive charges of this subdomain surface.

After leaving domain III, the L-chain runs alongside the calmodulin-like do-main IV in an extended conformation, presenting two clusters of acidic side chains toward the solvent (III-IV, Figure 1). At Ile530L, the L-chain enters the calmodulin-like domain IV, and, as had already been shown for the isolated (homodimeric) domains VI of rat and porcine m-calpain (Blanchard et al. 1997, Lin et al. 1997), this domain IV resembles other calcium-binding EF-hand proteins (Kretsinger 1996). It consists of eight α-helices connected through characteristic linkers, forming five EF-hand supersecondary structural elements 1 to 5.

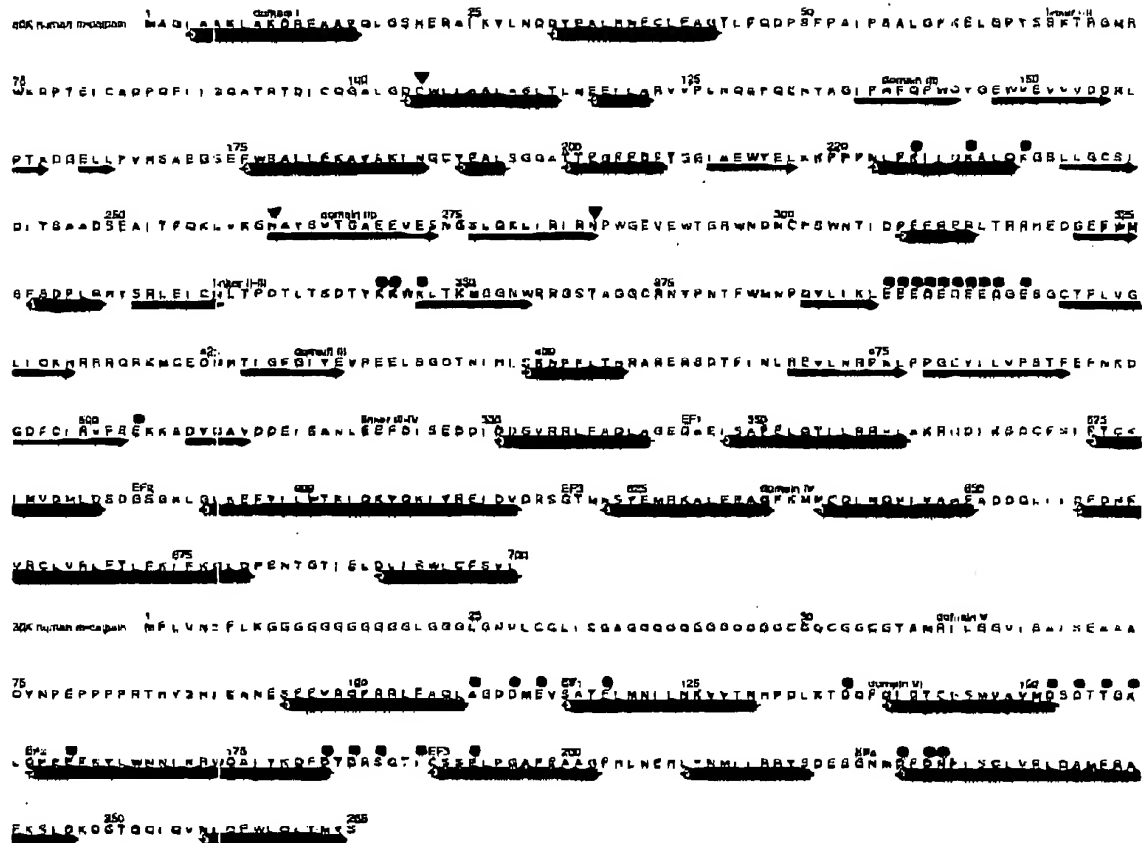The amino-terminal part of the m-calpain S-chain (Figure 1), rich in

Figure 2. Sequences of the 8(~kDa L- and the 30-kDa S-chain of human m-calpain (Imajoh et al. 1988, Ohno et al. 19h6). β-Strand, and α-helices are indicated by arrows and cylinders. Active-site residues and other notable residues (dIIa and dIIb) are marked by triangles, acidic residues of the s-itch loop (dIII) and opposing alkaline residues (dIIb and II-III), and the residues of domain VI known to be involved in normal calcium binding (Blanchard et al. 1997, Lin et al. 1997) by black circles. The figure was made with ALSCRIPT (Barton 1993)

bond, and S1, S2, etc. anc. S1', S2', etc. indicating the opposing subsites] should form a short antiparallel β-sheet with the enzyme, so that the (optimally Leu-, Val-, or Ile-like: Wang 2000) P2 side chain could slot into the relatively hydrophobic S2 depression. Consequently, the side chains of the P1, P1', and P2' residues would project out of the cleft running alongside the polar (S1) and positively charged rim (S2') of subdomain IIa, and the polar rim of subdomain IIb (S1'), respectively. Further, outside the cleft, several negatively charged side chains provided by both subdomains (increasing in number from μ- to m-calpain) would come into close contact with each other upon subdomain fusion (see Figure 3). It is tempting to speculate that without charge com-

pensation both charged subdomains could repel each other, preventing fusion. Calcium binding to any of these negatively charged sites may facilitate or even trigger subdomain fusion (Strobl et al. 2000). Such (hypothetical) calcium binding sites in the catalytic subdomains could explain the calcium sensitivity observed for the alternatively spliced nCL-2' (Table 1), that is, of a calpain species essentially consisting of the catalytic domain alone (Sorimachi et al. 1993). Based on sequence alignment and calcium binding studies alone, the His319L-Phe331L segment had previously been predicted as a "sixth" calpain EF-hand motif in domain III of conventional calpains (Andresen et al. 1991) However, the current structure reveals this helix-loop-strand-helix mo-

tive as a part of the catalytic subdomain IIb and differs from the typical EF-hand folds (Figures 1-3).

- Possible Effects Upon Calcium Binding to the Calmodulin-Like Domains

In a similar fashion to the rat dVI-dVI homodimer (Blanchard et al. 1997, Lin et al. 1997), the dIV-dVI heterodimer of human m-calpain should not fundamentally change its overall structure upon calcium binding to EF-hands 1, 2, and 3 (and EF-4 at higher calcium concentrations). More significant should be the refolding of the linkers involved in the three calcium-binding EF-hand motifs, allowing the acidic residue side chains involved to coordinate the bound cal-
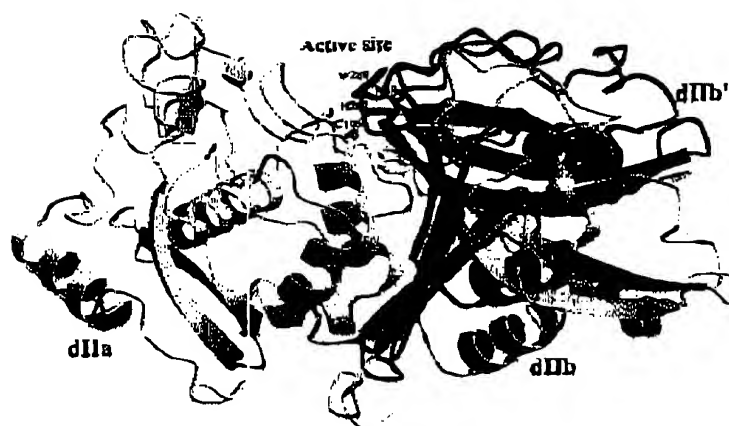
Figure 3. Superposition of the disrupted subdomains IIa and IIb of apo-m-calpain (dIIa and dIIb, pale grey) with the probable functional catalytic domain (dIIb', dark grey). Both helical subdomains superimpose (dIIa), while the barrel-like subdomain IIb has been arranged similar to papain (dIIb') The active site residues Cys105L, His262L, and Asn286L, as well as Trp288L are shown in full in the structure. This "standard view" of papain-like cysteine proteinases is obtained from Figure 1 by a 90° rotation around a horizontal axis. The figure was made with MOLSCRIPT (Kraula 1991) and Raster3D (Merrit and Bacon 1997).

cium ions. There is, however, no indication for a considerable exposure of new hydrophobic surfaces, so that domains IV and VI would appear to play a "structural" rather than a "regulatory" role in the conventional calpains.

Recent mutational studies on the EF-hand of rat m-calpain (Dutt et al. 2000) have shown that the importance of the EF-hand integrity for calcium activation varies. No single calcium binding site is absolutely required for calcium activation, and even an m-calpain species with all EF-hands mutated can be activated at higher calcium levels. The dissociation of the S- from the catalytic L-subunit of conventional human and rabbit calpain on exposure to calcium has been observed in several laboratories (Michetti et al. 1997, Suzuki and Sorimachi 1998, Vilei et al. 1997) but has been questioned by others (Elce et al. 1997). If correct, this observation would suggest a weakened interaction of both subunits upon calcium binding. The m-calpain L-subunit alone has been shown to be fully active (Vilei et al. 1997), so that the S-subunit seems to have a chaperone-like function for proper folding of the L-subunit.

Because of the presence of Asp154S in the EF2-hand, calcium binding to domain VI would disrupt the Lys7L-Asp154S

salt bridge and thus the interaction between the anchoring helix of the L-chain and the calmodulin-like domain VI, which would facilitate the release of the anchoring helix from its integrated original position (Figure 1). Consequently, this helix could adapt more easily to the substrate-binding sites of attacking proteinases or might even spontaneously unfold, allowing a rapid autolytic cleavage in helix dI (Suzuki and Sorimachi 1998), as frequently observed upon calcium activation. Such a release and irreversible truncation of the amino-terminal helix might mechanically relieve tension between domain VI and subdomain IIa, facilitating the latter's approach to subdomain IIb and the subsequent formation of a functional catalytic domain. Such a mechanism would be in agreement with the observed lower calcium requirement of autolyzed μ- and m-calpain (Suzuki et al. 1995).

• Possible Conformational Effects on Calcium Binding to Domain III

Owing to its central position, domain III may play a major role in the calcium-promoted activation of calpain (Hosfield et al. 1999, Strobl et al. 2000). Of particular interest is its surface-located extremely acidic loop, whose 10 negatively

charged side chains extend away from each other in apo-m-calpain, because of strong electrostatic repulsion (Figure 1). This loop directly contacts an amphipathic helix and the II-III connecting loop of subdomain IIb, which provide a number of basic (Lys) side chains (see Figure 2). These acidic and basic residues, as well as the corresponding negative and positive electrostatic potentials on both opposing (sub)domain surfaces, will certainly give rise to mutual attraction of subdomain IIb and domain III. Disruption of this electrostatic attraction should facilitate the fusion of subdomain IIb with subdomain IIa (Figure 4). In μ-calpain, with "only" eight negative charges distributed over a slightly longer loop segment, this charge repulsion should be slightly weaker, rendering electrostatic disruption easier. Indeed, it has been shown recently for rat m-calpain (Hosfield et al. 2001) that charge removal or inversion of one or the other charged residue in this interdomain region leads to an increased calcium sensitivity of the resulting calpain variants, indicating the importance of this electrostatic IIb-III contact for the transmission of triggering signals.

In our structural paper (Strobl et al. 2000), we furthermore speculated that positively charged particles such as calcium ions could bind to this acidic loop under (partial) charge compensation. Calcium binding could not only give rise to a more compact fold of this loop, but would also reduce its strongly negative potential and thus the electrostatic interaction with subdomain IIb. Such a calcium-induced disruption of the electrostatic interaction would allow subdomain IIb to rotate over to subdomain IIa and to fuse, under formation of a functional catalytic domain (Figure 4). The hydrophobic interactions between subdomain IIb and domain III would facilitate such a rolling motion of subdomain IIb, while subdomain IIa, clamped through many polar contacts, would remain rigidly oriented with respect to domain III (Figure 4).

Such an "electrostatic switch mechanism" (Strobl et al. 2000) would also account for the differences in calcium activation of μ- and m-calpain; m-calpain possessing the larger number of acidic residues in the acidic loop might coordinate more calcium ions than μ-calpain. Because of the stronger charge repul-
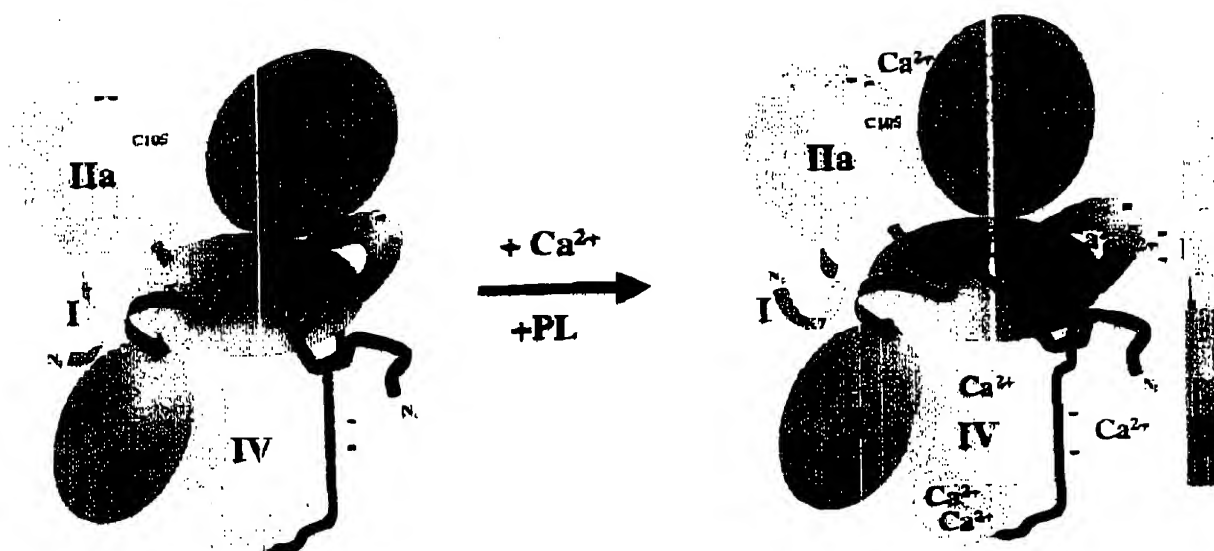
Figure 4. Schematic representation of the hypothetical calpain activation process on exposure to calcium and in the presence of phospholipids. Each m-calpain domain is represented by a sphere arranged as in Figure 1. Upon calcium binding to domain VI, the Lys 7L-Asp 154S salt bridge will be disrupted, giving rise to a liberation of the N-terminal domain dI and relieving any tension between dIU and dIIb. Calcium could bind to the negative charges on both sides of the active-site cleft, facilitating or even triggering subdomain fusion. Furthermore, upon calcium addition the acidic loop may fold around the calcium ions liberating the (mobile) subdomain IIb to form a functional catalytic domain with subdomain IIa. Such a dIIb movement is facilitated because of the hydrophobic base of dIII. Complete formation of the calcium coordination spheres of domain III may require additional ligands which are provided by the negatively charged phosphatidyl head groups of acidic phospholipids (PL).

sion of the more densely packed calcium ions, full charge compensation in the m-calpain acidic loop might require higher calcium concentrations. It is known that acidic phospholipids greatly reduce the calcium concentration necessary for calcium activation (Saido et al. 1992). The occupation of the calcium coordination spheres by acidic loop residues may remain incomplete, giving rise to the attraction of additional ligands such as oxygen from negatively charged phosphatidyl groups of membranous phospholipids (Strobl et al. 2000). Very recently, Tompa et al. (2001) were able to show that the isolated domain III of rat μ- and m-calpain binds calcium; and that the amount of bound calcium increases considerably in the presence of di- and triphosphoinositides-containing lisosomes. In the cell, the calcium ions might target and link the m-calpain molecules via domain III to specific cellular membranes.

A similar electrostatic switch mechanism, with a calcium-regulated approach of negatively charged target proteins and phospholipid membranes, had

been postulated for the C2-carrying synaptotagmin I involved in synaptic vesicle exocytosis (Rizo and Südhoff 1998). Calcium-mediated bridging between a two-calcium-binding C2 domain of protein kinase Cα and a phosphatidylserine molecule has recently been demonstrated crystallographically (Verdaguer et al. 1999).

Conclusions

Figure 4 shows schematically the potential calcium binding sites and possible calcium-promoted conformational changes, which may result in the formation of a functional and active catalytic domain. Calcium binding to the EF-hands of both calmodulin-like domains will presumably promote autolysis of the dI-helix and possibly ease the fusion of both catalytic subdomains, but should only "modulate" the overall (in)stability of the calpain molecule and calcium sensitivity of m-calpain, which is in agreement with the long distance from the catalytic subdomains IIa and IIb and with recent mutational experiments

(Dutt et al. 2000). Calcium binding to the multiple negative charges around the reformed active-site cleft, including the acidic residues in the former "sixth EF-hand" region, should further contribute to stabilization of the active calpain conformation. Recent mutational evidence (Hosfield et al. 2001) underscores the importance of the electrostatic interactions between the acidic tip area of domain III and the opposing alkaline region for transmission of the calcium-induced triggering signal toward the catalytic domain. This domain III, via its acidic loop, could be actively involved in this triggering event (Hosfield et al. 2001, Reverter et al. 2001, Strobl et al 2000, Tompa et al. 2001). In addition, it could mediate calpain's interaction with phospholipid membranes, increase its calcium sensitivity and direct the calpain molecule toward cytoplasmic and nuclear membranes. All the current data seem to suggest that in the conventional calpains the proteolytic activity is not regulated via a single switch, but that several sites cooperate in modulating the overall calcium response.

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Inherited Sodium Channelopathies: Novel Therapeutic and Proarrhythmic Molecular Mechanisms

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Voltage-gated sodium (Na) channels, transmembrane proteins that produce the ionic current responsible for the rapid upstroke of the cardiac action potential, are key elements required for rapid conduction through the myocardium and maintenance of the cardiac rhythm. The exquisite sensitivity of the cardiac rhythm to Na channel function is manifest in the proarrhythmic complications of "antiarrhythmic" Na channel blockade in patients with myocardial ischemia. More recently, studies of inherited single amino acid substitutions in Na channels have unveiled a remarkable array of cardiac rhythm disturbances, as well as surprising pharmacologic sensitivities. Hence, the sodium channelopathies are providing new molecular insights into mechanisms whereby altered ion channel behavior precipitates cardiac arrhythmias. (Trends Cardiovasc Med 2001;11:229–237). © 2001, Elsevier Science Inc.

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Inherited mutations in SCN5A, the gene encoding the human cardiac sodium (Na) channel, have been associated with varied disorders of cardiac rhythm that range from rapid, life-threatening tachyarrhythmias to bradyarrhythmias that require pacemaker implantation (Figure 1) (Schott et al. 1999, Wang et al. 1995). Na channels undergo rapid structural rearrangements on a sub-millisecond time scale in response to the changing transmembrane electrical field, through a process known as "gating" (Hodgkin and Huxley 1952). Whereas studies are linking the Na channel gating processes to particular structural elements, antiarrhythmic drug binding to cardiac Na channels hinges critically upon transitions among gated conformational states (Hille 1977, Hondeghem and Katzung 1977). It follows naturally that inherited Na channel mutations that provoke cardiac arrhythmias and gating dysfunction also seem to modify the clinical response to antiarrhythmic drug therapy (Fujiki et al. 1999, Schwartz et al. 1995) Will the emerging Na channelopathies serve as useful molecular models to understand how derangements in ion channel structure and function elicit more common, acquired rhythm disorders, such as those seen with coronary occlusion and structural heart disease? Moreover, do the inherited Na channelopathies provide a mechanistic framework for understanding unanticipated proarrhythmic responses to antiarrhythmic drug therapy?

• "Gain-of-Function" Gating Dysfunction: the Long QT Syndrome

As the myocardium is stimulated or "depolarized," the Na channels normally open only briefly (~1 msec), and then "fast inactivate" as the cardiac action potential ensues, producing a large inward current that rapidly extinguishes (Figure 2A). Mutations in the cardiac Na channel linked to an autosomal dominant form of the long QT syndrome ("LQT3," Figure 1; Wang et al. 1995) disrupt fast inactivation (Bennett et al. 1995, Dumaine et al. 1996, Wang et al. 1996) and thereby allow sustained channel opening. This evokes a small, persistent Na current during the action potential plateau (Figure 2A) that delays myocyte repolarization, evokes electrocardiographic (ECG) QT interval prolongation, and predisposes patients to polymorphic ventricular tachycardia ("torsade de pointes"). Surprisingly, the magnitude of this pathologic plateau current is minute (~0.5–2%) compared to the large "peak" Na current that develops immediately upon depolarization (Dumaine et al. 1996, Wang et al. 1996, Wei et al. 1999). The formidable role of this small "gain-of-function" defect in Na